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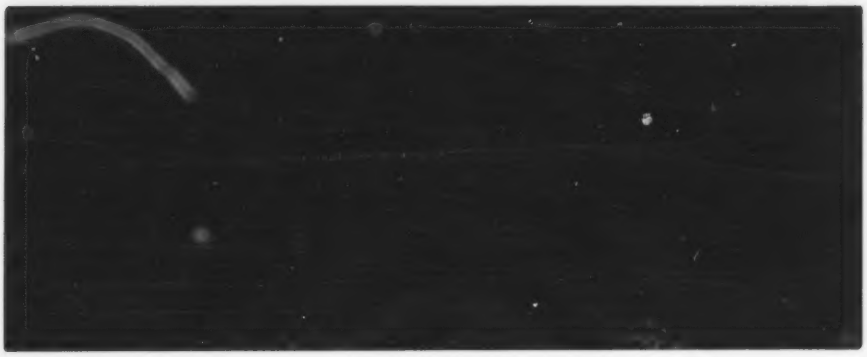

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Oxalate Content in Feeds and Its Metabolism by Ponies

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Title: Oxalate Content in Feeds and Its Metabolism by Ponies

Agriculturally, oxalic acid in plants is important as a toxic antagonist to divalent cations especially calcium. Oxalic acid is a dicarboxylic acid produced during normal plant growth. Total oxalate in plants represents a soluble portion as a potassium or sodium salt and an insoluble portion bound to calcium. Soluble oxalate may be absorbed and at high intakes is believed to cause acute hypocalcemia and death in ruminants by binding with calcium in body fluids. Insoluble oxalate binds with calcium in plant tissues and makes it inavailable for absorption. In alfalfa hay as much as 20-33% of calcium has been reported to be inavailable for digestion by cattle. Thus, insoluble oxalate can depress calcium availability and cause secondary calcium deficiency. A chronic calcium deficiency would be expected to cause bone abnormalities. Oxalic acid content in prairie feeds has not previously been considered high enough to be an important calcium antagonist.

Two hundred (200) feed samples were analyzed for total oxalate by a gas chromatographic method. Total calcium concentration was also measured. The results of the survery are given in Table 1. Legumes, mainly alfalfa hays, contained the highest calcium and oxalate concentrations ($P<0.05$) of all feedstuffs evaluated with the exception of one kochia hay which contained 3.2% oxalate, a level which would cause acute toxicity in cattle. Approximately one-half of the grass hay samples contained no oxalate. In grass samples positive for oxalate, the molar ratio of oxalate:calcium was 0.37. The molar ratio of oxalate to calcium was highest in native and domesticated grass and cereal hays. The oxalate:calcium ratio indicated that 36 to 44% of calcium could be potentially bound by oxalate in grass and cereal hays and about 25% of calcium could be bound in legume or legume-grass hays.

Available calcium was calculated by subtracting total molar oxalate concentration from total molar calcium content. The available calcium was determined to be greater than 1% in 80% of legume hays. Yet, the available calcium was below 0.2% in 33 to 62%

of grass, native and cereal hays in the study. Calcium concentrations less than 0.2% would not supply adequate calcium for maintenance of mature cattle or horses. Thus, oxalate antagonism is more important in forages normally low in calcium such as grass or cereal hays. These forages should be fed to livestock with inorganic calcium supplements.

Oxalate and calcium concentrations were measured in serially obtained samples from plot-grown grass and legume forages. None of the grass samples contained oxalate; alfalfa and sweet-clover contained 0.75 to 0.8% oxalate. The oxalate concentrations did not change in these forages throughout the growing season.

Digestion studies were conducted using ponies fed various forages. Calcium and oxalate balances were determined. Negative or low calcium balances were obtained when cereal hays were fed despite adequate calcium intakes. The negative calcium balance was attributed to an increase in calcium excretion in urine. Oxalate digestibilities were significantly higher from oat and barley hays than from alfalfa, brome or slough hays ($P<0.05$). This suggests that cereal hays contain more soluble oxalate than other forages and this might increase urinary calcium loss. These observations confirm the need for provision of adequate mineral supplementation when feeding cereal hays.

Agriculturally, the oxalate in prairie feeds is at a concentration that would be expected to cause secondary calcium deficiency. Oxalate concentrations that would cause acute toxicity in cattle were present in one kochia hay. Addition of inorganic calcium supplements to forage diets would compensate for calcium bound to oxalate.

Table 1. Average calcium and oxalate concentrations (% dry matter) and oxalate:calcium ratios in feedstuffs

Feedstuff	No.	Calcium	Oxalate	Oxalate:calcium Ratio
Legume	68	1.55	0.80	0.26
Legume-grass	30	1.15	0.63	0.25
Grass	58	0.55	0.14	0.22
Native grass	9	0.44	0.31	0.36
Cereal hay	24	0.37	0.33	0.44
Grain	4	0.09	0	0
Grain mixes	7	0.41	0	0
Kochia	1	0.80	3.20	1.77

OXALATE CONCENTRATION IN FEEDS AND ITS METABOLISM BY PONIES

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Total oxalate and calcium determinations were made in 200 feed samples. Legume hays contained more oxalate than legume-grass, grass, native grass and cereal hays ($P<0.05$). Grass, native and cereal hays contained low endogenous calcium and low to moderate oxalate concentrations. Based on molar ratios and the assumption of preferential binding of calcium by oxalate, it was estimated that about 25% of calcium in legume and legume-grass hays could be bound by oxalate. Similarly, oxalate might bind 38 to 44% of the calcium contained in native grass and cereal hays and exacerbate the low endogenous calcium content of these hays. Only one hay, a kochia, had oxalate concentrations (3.2%) known to cause acute toxicity in livestock. Oxalate concentrations in hays with low endogenous calcium may be important in creating secondary calcium deficiency. Oxalate concentration in feedstuffs was positively correlated to calcium concentration ($r=0.69$).

Oxalate and calcium concentrations were evaluated in plot-grown forages, 2 legumes and 3 grass hays, which were sampled at weekly intervals from immaturity to flowering. In plot-grown forage samples, only legumes contained detectable oxalate and its concentration did not change with plant maturity.

Chopped alfalfa, oat, brome, and slough hay and pelleted alfalfa, oat and barley hays were fed to ponies in 2 separate Latin square experiments. Apparent oxalate digestibilities were determined to be 54 to 88%. Cereal hays had the highest apparent oxalate digestibilities in both trials ($P<0.05$). Based on fecal oxalate, 10-15% of calcium may be irreversibly bound to oxalate. It was

hypothesized, however, that cecal-colonic microfloral digestion might occur and cause underestimation of calcium or other divalent cations bound to oxalate at the site of absorption.

INTRODUCTION

Agriculturally, oxalate is important as a toxicologic antagonist to divalent cations. Oxalic acid is an organic acid produced during normal plant growth (Dijkshoorn 1973). Oxalate accumulation in plants may depend on cation uptake and source of nitrogen used during growth (Franceschi and Horner 1980). Plant species, fertilization, precipitation, stage of growth and plant tissue can influence plant oxalate concentration (Blaney et al. 1981; Dijkshoorn, 1973; Seawright et al. 1970). Mold growth may also cause oxalate contamination of feeds (Wilson and Wilson 1961).

Acute toxicity from hypocalcemia or debility and death from chronic nephrosis have been recorded in cattle consuming pastures containing 6.9% oxalate (Seawright et al. 1970). However, chronic ingestion of moderate amounts of oxalate can lead to clinical bone disease in horses. Horses have developed osteodystrophia fibrosa grazing pastures marginally adequate in calcium but with calcium:oxalate ratios below 0.9 (Walthall and McKenzie 1976). Though ruminants have been considered tolerant of low intakes of oxalate, oxalate concentrations below 1.5% may be responsible for reducing calcium availability from alfalfa hay by 20-33% when fed to cattle (Ward et al. 1979). Recently, we reported oxalate concentrations ranging from 0.26 to 1.46% in various Saskatchewan forages and suggested that oxalate antagonism may have caused a reduction in calcium availability from cereal hays when fed to ponies (Cymbaluk and Christensen 1986). These results suggested that

oxalate accumulation occured in some prairie forages and that oxalate merited consideration as a calcium antagonist.

The objectives of this study were to quantitate oxalate concentrations in prairie forages and to evaluate the metabolism of forage oxalate by ponies.

MATERIALS AND METHODS

Methodology for Oxalate Quantitation. The method of Roughan and Slack (1977) was modified for oxalate determination in feed, feces and acidified, freeze-dried urine. The main modification to the method was the use of a longer column which facilitated use of smaller sample injections and a shortened run time. A Perkin-Elmer Sigma 2000 gas chromatograph fitted with a 30 m Durabond Wax column was used for analysis. Peak areas were determined on a Hewlett Packard 3390A integrator.

Calcium Quantitation. Calcium in feeds and feces was determined by atomic absorption spectroscopy after acid digestion of samples (Zasoski and Burau 1977). Urinary calcium was determined directly after appropriate dilution with 0.5% lanthanum oxide solution .

Sample Preparation. Feeds and feces were dried in a forced air oven at 65°C for a minimum of 48 h. Samples were ground using a Wiley mill to pass through a 5.0-mm screen. Then 0.200 g sample was placed in a 16 mm glass test tube fitted with a plastic cap and 5 ml of extraction methylation solution (Roughan and Slack 1973) was added.

The sample was vortexed then incubated overnight at room temperature. One ml of water was added, then the mixture was vortexed and filtered through phase separating filter paper. A 0.2-µl aliquot of the filtrate was injected for oxalate quantitation. Acidified urine samples (2 ml) were freeze-dried. The remaining pellet was processed in the same way as feed and fecal samples. Recoveries of oxalate by this method were examined by adding oxalic acid standards to oat hay and equine fecal samples.

Oxalate Concentrations in Feeds. Two hundred feed samples were obtained from the Saskatchewan Feed Testing Service and from Alberta Agriculture. The majority of samples were forages but 11 grain or grain mixes were also evaluated. Legume hays were predominantly alfalfa. Legume-grass hays were alfalfa-brome or alfalfa-timothy. The proportions of legume to grass were not recorded in most instances. Cultivated grass hays included timothy and brome hays. Native grass hays were prairie or slough hay. Cereal hays included oat, barley, and fall rye but were mainly oat hay. One kochia, (Kochia scoparia), was also analyzed. Each sample was analyzed in duplicate.

Changes in oxalate concentration of forages with advancing maturity were assessed in five forages grown in duplicated 125-150 m^2 plots (Agriculture Canada, Saskatoon). The forages analyzed were Beaver alfalfa, Yukon sweet clover, Regar bromegrass, Parkway crested wheat grass, and Greenleaf intermediate wheat grass. Random samples (500 g) were taken starting on May 28, 1984 then at 7 d

intervals to July 4 and on July 16, 1984. All forage samples collected on June 11 were separated into leaf and stem portions and analyzed for calcium and oxalate content. These samples were freeze-dried.

Digestion Studies. A 4 x4 Latin square experiment using pony geldings was used to evaluate chopped alfalfa, brome, oat and slough hay digestibility. A 3 x 3 Latin square experiment was similarly conducted to evaluate pelleted alfalfa, oat and barley hays. Total fecal and urinary collections were made for 6 days following adaptations periods of 21 days. Calcium and oxalate determinations were made on feed, fecal and urine composites.

Analyses of data included descriptive statistics, analysis of variance, Student-Neuman-Keuls' mean comparisons and linear regression analysis (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

The fused silica column produced a chromatogram containing three, and occasionally four, main peaks in the 7 to 10 min run time for methylated feeds and fecal samples. The two most rapidly eluted peaks were solvents. Dimethyloxalate was eluted at 2.88 min and the fourth peak, dimethylhydroxybutanedioic acid, eluted at 3.17 min. The latter peak occured in a limited number of samples. Confirmation of peak composition was based on methylated oxalic acid standards and gas chromatographic mass spectrometry. The coefficient of variation for repeated analyses of samples containing 0.34, 0.52 and 1.07% oxalate were 14.7, 7.7 and 4.4%, respectively. The standard

deviation of determinations within samples was 0.05 indicating that the error in repeated analyses of the same sample was probably variation in injection technique. Recoveries of added oxalate to samples with known concentrations ranged from 103 to 123%.

Oxalate Concentrations in Feeds. The mean, standard deviation and range of feedstuff calcium and oxalate and the molar ratio of oxalate to calcium are given in Table 1. Legumes contained the highest calcium and oxalate concentrations ($P<0.05$). Calcium concentrations were typical for Saskatchewan and Alberta forages (Christensen et al. 1980; Suleiman and Weisenburger, 1983). The molar ratio of oxalate to calcium was used rather than the calcium:oxalate ratios given by others (Blaney et al 1981; Hintz et al. 1984) since this more clearly defines the percentage of calcium potentially bound by oxalate. For the 68 legume samples, the mean oxalate:calcium ratio The oxalate:calcium ratio for all grass hay samples was 0.22 but for grasses positive for oxalate the ratio was 0.37. In these latter samples, one-third of the total calcium could be unavailable.

The mean calcium and oxalate concentrations of the nine native grass hays were 0.44 and 0.31%, respectively. The oxalate:calcium ratio was 0.36 indicating a possible low calcium availability from these forages. Cereal hays were similar in both calcium and oxalate concentrations to native hays. The oxalate:calcium ratio was 0.44 for cereal hays. Grains and grain mixes contained no detectable oxalate. The high calcium concentration of grain mixes was due to added mineral supplement.

Only one kochia sample was submitted during the survey and was remarkable in its high oxalate concentration (3.2%).The oxalate:calcium ratio was 1.77 suggesting that all calcium could be bound to oxalate. Kochia has been reported to be an oxalate accumulator (Cheeke and Shull, 1985). Oxalate content of kochia should be further appraised since acute hypocalcemia would be expected at these concentrations in unadapted ruminants.

Franceschi and Horner (1980) have reported that oxalate production may be related to cation balance, especially calcium, within plant tissue. In the present study, the relationship of % oxalate concentration (Y) to % calcium concentration (X) was determined to be linear according to the equation, $Y= 0.42X + 0.09$ ($r=0.69$; $P<0.01$) in 193 forage and grain samples. Grain mixes were not included since calcium concentration was altered by addition of mineral supplements.

Oxalate in plant tissues can exist as a soluble potassium or sodium salt or as an insoluble calcium crystal (Franceschi and Horner 1980). Insoluble oxalate can comprise 23 to 86% of total oxalate in tropical grasses (Blaney et al. 1981). The available calcium in forages of this survey was estimated by subtracting the molar concentration of oxalate from the molar concentration of total calcium and assumes that oxalate binds exclusively to calcium. The frequency of samples containing available calcium below 0.2, 0.5 and 1.0% in the forage evaluated in this survey are given in Table 2. The value of 0.2% was chosen as representing the minimum calcium requirement for mature horses and cattle; 0.5% was considered the

minimum needed by yearling horses and weanling cattle if forages were fed exclusively (NAS-NRC 1978a; 1978b). A 1% calcium level was chosen as a reference where forages if fed at the rate of 50% of the diet could be used as the sole source of calcium for young livestock.

Only 20% of legume samples contained less than 1.0% available calcium and only 3% were below 0.5%. Few (7%) legume-grass hays had estimated available calcium below 0.2% and only 23% of these hays were estimated to have less than 0.5% available calcium. By comparison, 69% of grass hays supplied less than 0.5% calcium and 38% supplied less than 0.2% available calcium. In the latter category, 54% had less than 0.2% calcium without exacerbation by oxalate. Native grass hays had a similar available calcium distribution as cultivated grass hays. Cereal hays were predicted to be the most limiting in available calcium. Available calcium was estimated to be below 0.2% in 62.5% of the samples. Only 1 of 24 samples was below 0.2% prior to correction for oxalate content but 92% of cereal hays had total calciums below 0.5%.

Native, cereal and some grass hays had low total calcium concentrations relative to legumes. Furthermore, the oxalate in native and cereal hays may bind as much as 36 to 44% of total calcium. Thus these forages must be considered poor calcium sources for livestock. These hays when fed to horses and young cattle should be fed with a supplementary calcium source.

Oxalate in Forages with Plant Maturity. None of the plot-grown grass species examined contained detectable oxalate. Poor growing conditions including lack of moisture and fertilization may have contributed to the absence of oxalate in grass hays. Sweet-clover and alfalfa were positive for oxalate. The changes in calcium and oxalate concentrations over time are given in Fig. 1. Calcium and oxalate concentrations did not change in alfalfa over the sampling period, May 28 to July 16, 1984. Although oxalate concentrations in sweet clover did not change, calcium concentrations in this forage declined steadily over the growing period. The mid-July sweet-clover sample was noted to be in very advanced maturity and senescence.

The distribution of calcium and oxalate in early bud alfalfa and budding sweet-clover stem and leaves are given in Table 3. Calcium and oxalate concentrations are millimolar. Feed calcium and oxalate concentrations of 1% would be equivalent to 25 and 11.1 mM/100 g DM. The molar equivalence of oxalate to calcium in whole plant and leaf was 0.19 and 0.22, respectively, but 0.05 for stems. Sweet clover whole plant, leaf and stem had oxalate:calcium ratios of 0.19, 0.27 and 0.06, respectively. The values obtained for whole plant and leaves were similar to those derived by Ward et al. (1979) for alfalfa hays. The oxalate:calcium ratio of stem was about one-quarter of the leaf portions and indicates that oxalate is accumulated in leaves. Calcium oxalate crystals have been identified to occupy bundle sheath cells in alfalfa leaves but to be less frequently present and less well organized in stem (Ward et al. 1979). McKenzie and Schultz (1983) reported that calcium oxalate

crystals in grasses could vary in density among leaves within the same plant.

Oxalate Digestion by Ponies. Calcium digestibilities are given in Table 4. True calcium digestibilities were calculated using 20 mg calcium/kg body weight as the endogenous fecal calcium excretion for horses (Schryver et al. 1972). Calcium digestibilities from alfalfa and brome hays were comparable and higher than from slough and oat hays ($P<0.05$). The lowest calcium retention was observed when oat hay was fed. True calcium digestibilities were comparable for pelleted alfalfa and barley hays and were higher ($P<0.05$) than from oat hay pellets. Ponies were in negative calcium balance when both cereal hays were fed.

Oxalate balance by ponies fed chopped or pelleted forages are given in Table 4. The apparent digestibility of oat hay oxalate was higher than for alfalfa, brome and slough hays ($P<0.05$). Pelleted cereal hays had higher apparent oxalate digestibilities than pelleted alfalfa hay ($P<0.05$). The apparent oxalate digestibilites obtained here were similar to values of 66.5 to 94.7% obtained by Blaney et al. (1981) for horses fed forage diets containing 0.18 to 3.74% endogenous oxalate.

The main route of oxalate excretion was feces. Assuming fecal oxalate represents insoluble calcium oxalate, then the molar concentration of oxalate can be used to predict calcium which was unavailable for absorption during gastrointestinal transit. The inavailable calcium (bound to oxalate) in feces as a % of total

calcium intake would be 15, 13, 7.2 and 8.1%, respectively, in chopped alfalfa, brome, oat and slough hays. This is lower than the 20 to 33% calcium predicted to be unavailable for absorption by ruminants fed alfalfa (Ward et al. 1979).

However, fecal oxalate is likely not representative of the soluble oxalate complexes formed during small intestinal transit. Ward and Harbers (1982) showed that only 3% of oxalate and 65 to 70% of calcium are available in solutions at a pH above 2.5. The pH of the stomach and post-gastic intestine of ponies have been reported to exceed 2.5 and 5, respectively (Argenzio et al. 1974). Thus, the pH of the equine small intestine would promote calcium oxalate precipitation. Yet, fecal oxalate may be much lower than the actual insoluble oxalate complexes in the small intestine. Post-ileal microfloral degradation of oxalate must be considered in horses since rumen microflora has shown to adapt and catabolize oxalate substrates (Allison et al. 1977). Passive diffusion from the colon has been reported to be a mode of oxalate absorption in man (Williams and Smith 1983) and could also occur in the horse. From the data of Blaney et al (1981), the digestibility of insoluble oxalate by horses can be inferred to be between 28 to 86% and suggests that insoluble oxalate is not inert to digestive processes.

Oxalate excretion in urine was minimal by ponies. Blaney et al (1981) observed a positive increase in urinary oxalate as intake increased and obtained values of 0.5 to 6.5 mg oxalate/ kg body weight. Ponies excreted 3 to 7 mg oxalate/kg body weight in urine in the present study. Thus, normal horses on forage diets do not appear

to use renal detoxification as a mode of oxalate elimination. Absorbed oxalic acid in man has been noted to be eliminated primarily in urine (Williams and Smith 1983) although Prenen et al (1984) found little change in urinary oxalate concentration in man fed oxalate-rich diets. We obtained values of only 2.5 to 12.6% urinary excretion of absorbed oxalate.

Oxalate metabolism in animals has not been fully explained. Endogenous oxalate production can arise from metabolism of ascorbate, amino acids and glycolate (Williams and Smith 1983). However, the toxic nature of oxalate is the agriculturally important consideration. Oxalate toxicity can occur in ruminants following sudden excessive intakes (Seawright et al. 1970) and this is largely attributable to soluble oxalate. Chronic low intakes of oxalate may also cause metabolic bone disease in horses (Walthall and McKenzie 1976) and its role in this capacity has not been evaluated in ruminants. The chronic component is likely associated with the formation of insoluble calcium oxalate and would be related to production of marginal or subnormal calcium absorption. Blaney et al. (1982) have shown that calcium absorption is reduced by 20% when cattle are fed moderate concentrations of sodium oxalate. The low to moderate oxalate content of prairie forages would not be expected to cause acute toxicities but in certain forage species might aggravate marginal calcium intake thereby causing subclinical bone disease. Generally, grass and cereal hays should be viewed as forages with a poor endogenous calcium supply. Use of inorganic calcium supplementation would be advised when feeding these forages,

especially to young livestock. Oxalate concentration in legume hays should also be evaluated. Mineral supplementation may be adviseable in dairy cattle diets in which alfalfa is provided as the sole source of calcium if the oxalate concentrations are elevated.

IMPACT

Total oxalate is comprised of soluble and insoluble oxalate. Soluble oxalate is thought to be absorbed and may cause acute toxicity by binding calcium in body fluids. Insoluble oxalate has been considered to be the calcium oxalate crystals formed in plant tissues and calcium bound to oxalate in this form would be poorly digestible to indigestible. Thus, oxalates depending on their content in plant tissues can cause sudden death or impair calcium uptake and produce secondary calcium deficiency. In animals, the latter would cause bone abnormalities.

Antagonism of calcium absorption by oxalates has not previously been considered important in prairie grown forages. Our results indicate that oxalate is present in prairie forages. Oxalate concentrations were highest (0.8%) in legume hays, mainly alfalfa. The oxalate concentrations in forages could potentially bind from 25 to 44% of the calcium contained in legume and grass hays. Oxalate was not present in cereal grains. The highest oxalate concentration obtained was 3.2% in a kochia hay sample. This concentration would cause acute toxicity in cattle.

Ponies digest from 46 to 88% of oxalate contained in forages. The indigested oxalate would be predicted to bind with calcium in the gut and make this calcium inavailable. The lowest oxalate digestibility was from alfalfa hay; the highest from cereal hays. Negative calcium balance was observed when cereal hays were fed. Calcium content of cereal hays was marginally adequate for maintenance. The negative calcium balance suggests that oxalate may

have impaired calcium absorption and increased excretion of calcium in the urine.

Oxalate antagonism to calcium absorption may be most important in forages that normally have a low calcium supply such as grass and cereal hays. These forages should be fed with supplemental calcium particularly when fed to young cattle or horses.

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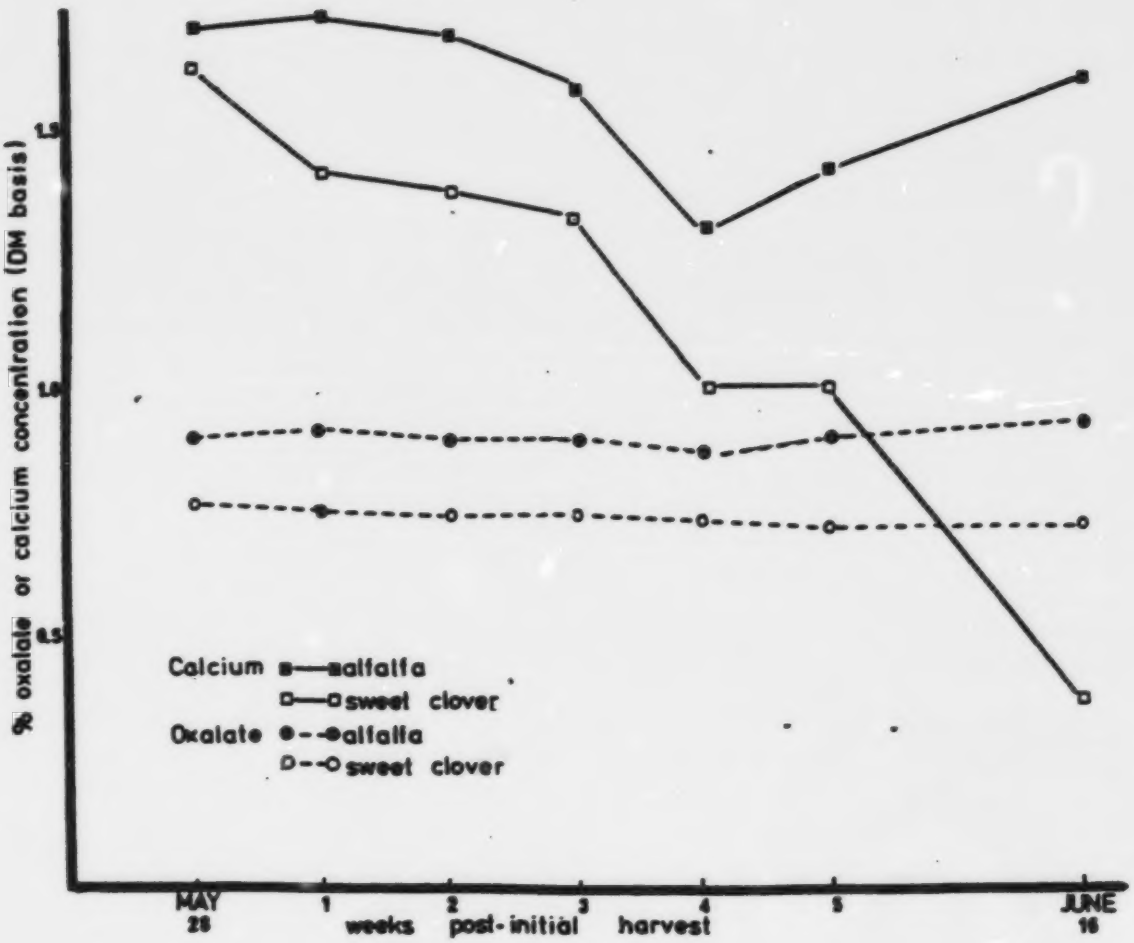

Figure 1. Changes in oxalate and calcium concentrations (% dry matter) in early bud alfalfa and budding sweet clover.

Table 1. Calcium and oxalate concentrations (% dry matter) and molar ratios of oxalate:calcium in feedstuffs

Feedstuff	No.	Calcium			Oxalate			Oxalate:calcium		
		Mean	S_d	Range	Mean	S_d	Range	Mean	S_d	Range
Legumes	68	1.55*a*	0.41	0.37-2.98	0.83*a*	0.27	0.34-2.98	0.26*ab*	0.12	0.09-0.87
Legume-grass	30	1.15*b*	0.50	0.10-2.3	0.60*a*	0.42	0-1.85	0.25*ab*	0.15	0-0.68
Grass	58	0.55*c*	0.32	0.10-1.64	0.14*c*	0.18	0-0.81	0.22*a*	0.21	0-0.60
Native Grass	9	0.44*c*	0.15	0.26-0.69	0.31*b*	0.10	0.20-0.51	0.36*ab*	0.16	0.13-0.65
Cereal Hay	24	0.37*c*	0.14	0.14-0.79	0.33*b*	0.13	0.19-0.66	0.44*b*	0.20	0.14-0.74
Grains	4	0.09	0.05	0.05-0.12	0	0	0	0	0	0
Grain Mixes	7	0.41	0.10	0.31-0.54	0	0	0	0	0	0
Kochia	1	0.80	-	-	3.20	-	-	1.77	-	-

a-c Values with the same letter in the same column are different ($P<0.05$). Grains and kochia were not used in mean comparisons.

Table 2. Estimated available calcium of forages (% frequency)

Forage	No.	Available Calcium <0.2%	<0.5%	<1.0%
Legume	68	1.5	2.9	20.6
Legume-grass	30	6.7	23.3	56.7
Grass	58	37.9	69.0	91.4
Native grass	9	33.3	88.9	100.0
Cereal	24	62.5	91.7	100.0

Table 3. Calcium and oxalate concentrations (mM/100 g DM) and oxalate: calcium ratios in early bud alfalfa and budding sweet clover

	Calcium	Oxalate	Oxalate:calcium Ratio
Alfalfa			
whole plant	41.2	9.0	0.22
leaf	48.2	9.1	0.19
stem	24.7	1.2	0.05
Sweet Clover			
whole plant	31.4	8.6	0.27
leaf	44.2	8.6	0.19
stem	21.0	1.3	0.06

Table 4. Daily calcium balances (g/100 kg BW) in ponies fed forages

Forage	Intake	Feces	Apparent Absorbed	Urine	Retained	% True Calcium Digestibility §
Trial 1						
Alfalfa	23.2*a*	8.0*a*	15.2*a*	6.4*a*	8.8*a*	74.1*a*
Brome	12.6*b*	5.7*b*	6.9*b*	3.7*b*	3.2*b*	69.5*b*
Oat	6.8*d*	5.0*b*	1.8*d*	1.0*c*	0.8*d*	55.3*c*
Slough	9.3*c*	5.6*b*	3.7*c*	2.2*b*	1.5*c*	61.7*b*
SEM †	1.7	0.4	1.4	0.6	0.7	2.3
Trial 2						
Alfalfa	23.5*a*	7.8*a*	15.7*a*	8.5*a*	7.2*a*	74.8*a*
Oat	5.3*b*	3.8*b*	1.5*b*	2.0*b*	-0.5*b*	63.7*b*
Barley	4.7*b*	3.2*b*	1.5*b*	1.8*b*	-0.3*b*	71.2*a*
SEM †	2.2	0.5	1.7	0.8	0.9	3.5

a-d Values within a column and comparison with the same letter are not different at $P<0.05$.

† Standard error of mean.

§ Calculated as true digestibility (see text).

Table 5. Daily oxalate balances (g/100 kg BW) in ponies fed forages

Forage	Intake	Feces	Apparent Absorbed	Urine	Retained	% Apparent Digestibility
Trial 1						
Alfalfa	20.9*a*	7.9*a*	13.0*a*	0.5	12.5*a*	62.4*a*
Brome	10.2*b*	3.7*b*	6.4*b*	0.7	5.2*b*	63.2*a*
Oat	9.2*b*	1.1*c*	8.2*c*	0.7	7.6*c*	88.1*b*
Slough	4.2*c*	1.7*c*	2.6*d*	0.3	2.3*d*	65.9*a*
SEM†	1.6	0.7	1.0	0.1	1.0	3.9
Trial 2						
Alfalfa	15.0*a*	7.8*a*	6.9*a*	0.7	6.1*a*	45.9*a*
Oat	5.5*b*	1.4*b*	4.1*b*	0.6	3.5*b*	74.3*b*
Barley	3.7*c*	1.0*b*	2.7*c*	0.6	2.1*b*	73.3*b*
SEM†	1.8	1.1	0.6	0.1	0.6	4.8

a-d Values with the same letter in the same comparison are not different ($P<0.05$).

† Standard error of mean.